



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<p>(51) International Patent Classification ⁵ : A61K 31/70</p>	<p>A1</p>	<p>(11) International Publication Number: WO 95/28940 (43) International Publication Date: 2 November 1995 (02.11.95)</p>
<p>(21) International Application Number: PCT/US94/04436 (22) International Filing Date: 22 April 1994 (22.04.94) (71) Applicant (for all designated States except US): DEPARTMENT OF THE ARMY [US/US]; John Moran, Off. of Command Judge Adv., HQUSAMRDC, Fort Detrick, Frederick, MD 21702-5012 (US). (72) Inventors; and (75) Inventors/Applicants (for US only): CHIANG, Peter, K. [US/US]; Walter Reed Army Institute of Research, Washington, DC 20307-5100 (US). MAYERS, Douglas, L. [US/US]; Walter Reed Army Institute of Research, Washington, DC 20307-5100 (US). BURKE, Donald, S. [US/US]; Walter Reed Army Institute of Research, Washington, DC 20307-5100 (US). (74) Agent: HENDRICKS, Glenna; 9669 A Main Street, Fairfax, VA 22031 (US).</p>		<p>(81) Designated States: CA, JP, US, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE). Published <i>With international search report.</i></p>
<p>(54) Title: METHODS FOR INHIBITING HUMAN IMMUNODEFICIENCY VIRUS</p>		
<p>(57) Abstract</p> <p>Pharmaceutical formulations of neplanocin-A, 3-deazaneplanocin, 3-deazaadenosine, 4'-thioadenosine and 5-azacytidine wherein homocysteine or homocysteine lactone are additional components are disclosed along with methods of treating HIV with same. In addition, the same five antiviral agents are disclosed to form complexes with cyclodextrin with utility in the treatment of HIV in human hosts.</p>		

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	GB	United Kingdom	MR	Mauritania
AU	Australia	GE	Georgia	MW	Malawi
BB	Barbados	GN	Guinea	NE	Niger
BE	Belgium	GR	Greece	NL	Netherlands
BF	Burkina Faso	HU	Hungary	NO	Norway
BG	Bulgaria	IE	Ireland	NZ	New Zealand
BJ	Benin	IT	Italy	PL	Poland
BR	Brazil	JP	Japan	PT	Portugal
BY	Belarus	KE	Kenya	RO	Romania
CA	Canada	KG	Kyrgyzstan	RU	Russian Federation
CF	Central African Republic	KP	Democratic People's Republic of Korea	SD	Sudan
CG	Congo			SE	Sweden
CH	Switzerland	KR	Republic of Korea	SI	Slovenia
CI	Côte d'Ivoire	KZ	Kazakhstan	SK	Slovakia
CM	Cameroon	LI	Liechtenstein	SN	Senegal
CN	China	LK	Sri Lanka	TD	Chad
CS	Czechoslovakia	LU	Luxembourg	TG	Togo
CZ	Czech Republic	LV	Latvia	TJ	Tajikistan
DE	Germany	MC	Monaco	TT	Trinidad and Tobago
DK	Denmark	MD	Republic of Moldova	UA	Ukraine
ES	Spain	MG	Madagascar	US	United States of America
FI	Finland	ML	Mali	UZ	Uzbekistan
FR	France	MN	Mongolia	VN	Viet Nam
GA	Gabon				

METHODS FOR INHIBITING HUMAN IMMUNODEFICIENCY VIRUS

Field of the Invention:

This invention relates to use of neplanocin-A, 4'-thio-adenosine, 5-aza-cytidine, 3-deaza-aristeromycin and 3-deaza-neplanocin for inhibition of human immunodeficiency virus (HIV), especially when the virus is resistant toward 3'-azido-3'-deoxythymidine (AZT).

Background of the Invention:

The treatment of AIDS using antiviral medication has met with minimal success. The use of 3'-azido-3'-deoxythymidine (AZT) for treatment has proven problematical for several reasons. Virus exposed to AZT often develop a resistance to AZT. Furthermore, AZT is a very toxic agent. Long term treatment often results in anemia associated with erythroid hypoplasia and megaloblastic changes in the bone marrow. The drug itself is expensive and the cost of laboratory monitoring of patients results in still further expense. Additional effects of AZT include severe headache, nausea, insomnia and myalgias. It is essential that new agents be found that will effectively inhibit AZT-resistant strains and are devoid of toxic reactions.

The effectiveness of an antiviral agent against other viruses is not predictive. Several antiviral agents which are effective against other viral infections have proven to be ineffective against HIV, and AZT is not effective against such viruses as herpes simplex or varicella.

Dideoxyinosine (ddI) and dideoxycytidine (ddC) have also been shown to be useful in the treatment of AIDS. Dideoxyinosine has presently been approved for clinical use. The primary toxic effects are primarily peripheral neuropathy and pancreatitis. When given in conjunction with AZT some of the more serious effects of both drugs can be minimized. The drug ddC has shown excellent antiviral activity and is presently undergoing clinical trials. Currently it has been shown to

present a pattern of peripheral neuropathy similar to that of ddI. However, pancreatic effects appear to be less serious than effects seen using ddI.

Neplanocins have been known previously to have antitumor activity. Some of the neplanocins, including A and C, have also been known to have growth inhibitory activity on some kinds of plant pathogenic fungi. Neplanocin-A has also been reported to have antimalarial activity. (Whaun, et al., Journal of Pharmacology and Experimental Therapeutics, Vol. 236, No. 1, pp 277-282 (1986)) There is no suggestion that neplanocin-A would inhibit HIV.

Adenosine analogues as substrates and as inhibitors of S-adenosylhomocysteine hydrolase, including carbocyclic adenosine, are taught by Guranowski, et al. (Biochemistry, Vol. 20, No. 1, pp 110-155 (1981)). There is no teaching regarding neplanocin-A therein. Antiviral properties of neplanocin-A and its analogs are described by DeClerq, et al. (Antimicrobial Agents and Chemotherapy, Vol. 33 No. 3, pp 1291-1297 (1989)). De Clerq comes to the conclusion that neplanocin-A is not effective against HIV at non-toxic levels.

3-Deaza-aristeromycin (also known as carbocyclic 3-deaza-adenosine) is disclosed in U.S. Patent 4,386,093 to Chiang, et al which is incorporated herein by reference, has been shown to be effective for inhibiting herpes simplex virus. There is no suggestion therein that 3-deaza-aristeromycin would be effective against HIV. The 3-deaza-aristeromycin is relatively non-cytotoxic at antiviral concentrations and is not subject to deamination or phosphorylation. At a K_i of $3 \times 10^{-6}M$ it acts as a competitive inhibitor of S-adenosylhomocysteine hydrolase. Wyde, et al. (Antiviral Research, 14 (1990) 215-226) discuss the antiviral efficacy of 3-deaza-aristeromycin against respiratory syncytial virus (RSV) and parainfluenza type 3 virus (PIV3) infections when tested in tissue culture and in cotton rats. In cotton rats, animals given 1 mg/kg/day intraperitoneally showed consistent reductions in disease when compared to control animals. No toxic effects were noted in cotton rats, even in animals given 20 mg/kg/day for eight

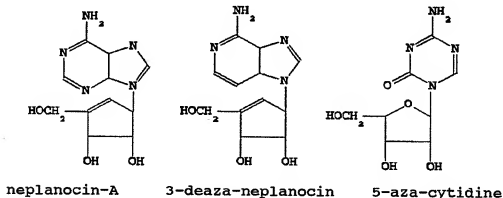
consecutive days. However, there is no indication therein that 3-deazaaristeromycin might be effective against HIV infection.

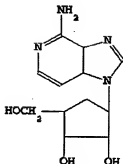
The discussion of carbocyclic analogs of 3-deaza-adenosine, 3-deaza-aristeromycin and 3-deazaneplanocin, are described by Chiang, et al as inhibitors and as alternative substrates of S-adenosylhomocysteine hydrolase (Journal of Biological Chemistry, Vol.287, No. 7, pp 4988-4991 (1992)). There is no suggestion therein that either compound has use for inhibition of HIV.

Detailed Description of the Invention:

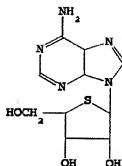
It has now been found that neplanocin-A, 4'-thioadenosine, 5-aza-cytidine 3-deaza-aristeromycin and 3-deazaneplanocin are very effective HIV inhibiting agents. Even more surprising is the discovery that these compounds are particularly effective against HIV that have been shown to be AZT resistant. The use of the particular species for purposes of inhibiting the activity of the AIDS virus is particularly important in the fight against AIDS in persons who have been treated with AZT and either no longer respond to treatment or are suffering from unacceptable side effects.

Agents for use in accord with the teachings of the invention are the following:





3-deaza-aristeromycin



4'-thio-adenosine

While amino and hydroxy groups may be carboxylated to provide esters and amides, the unsubstituted compounds are believed to be most useful for providing immediate beneficial results against the HIV virus.

The compositions have been studied in accord with the methods described below.

Viral Isolation: All clinical HIV-1 isolates were obtained by co-cultivation of phytohemagglutinin (PHA)-stimulated donor peripheral blood mononuclear cells (PBMC) with fresh patient PBMC obtained by ficoll-Hypaque separation of heparinized blood. Isolates were expanded on fresh donor PBMC to obtain a virus stock which was frozen in aliquots at -180°C .

Viral Stock Titration: ACTG/DOD Consensus HIV-1 Drug Susceptibility Assay: The tissue culture infectious dose 50% (TCID_{50}) was determined by endpoint dilution using sextuplicate serial 4-fold dilutions ranging from 1:16 to 1:64,000 in a 96-well microtiter plate. Each well contained 2×10^5 PHA-stimulated donor PBMC in a total volume of 200 μl of supplemented RPMI-1640. Plates were incubated at 37°C in humidified air with 5% CO_2 . On the fourth day, the cells were resuspended, split 1:3, and fresh medium was added. On the seventh day, 100 μl of supernatant was assayed for p24 antigen. Wells were scored as positive if p24 Ag was $> 30 \text{ pg/ml}$. One TCID_{50} was defined as the amount of virus stock at which 50% of the inoculated wells were positive and was based on the number of positive wells at each viral dilution using the Spearman-Kärber method.

ACTG/DOD HIV-1 Drug Susceptibility Assay: PHA-stimulated donor PBMC were incubated with cell-free virus stock at 200 TCID₅₀ per 2 X 10⁵ cells for one hour at 37°C. The cells were washed with RPMI-1640 and centrifuged at 300 g for 10 minutes. The supernatant was removed and the cells were resuspended in 8.0 ml of fresh medium. A 96-well microtiter plate was set up with 2 X 10⁵ cells/well added to drug containing media with final concentrations of 0.001, 0.01, 0.1, 1.0, and 5.0 µM zidovudine (AZT). The concentration of 3-deazanucleosides used in this study were 0.001, 0.01, 0.1 and 1.0 µM. There were six no-drug control wells and triplicate wells for each drug concentration. Co-cultures were incubated at 37°C in humidified air with 5% CO₂.

On the fourth day the cells were resuspended and split 1:3. Media with the appropriate drug concentration was replaced in each well and the plates were returned to the incubator. Supernatant p24 Ag was quantitated at day seven by antigen capture ELISA (Coulter Immunology). The 50% inhibitory concentration (IC₅₀) for each drug was determined by comparing the p24 antigen values in the control wells to the values in the drug containing wells using the median effect equation.

Cytotoxicity Studies: Into wells of a microtiter plate were placed 2 X 10⁵ PHA-stimulated normal PBMCs and cultured media (200 µl) with various concentrations of each drug. Cells in control wells were cultured in media without a drug. On the fourth day, the cells in the wells were mixed and 125 µl of media was removed and 150 µl of fresh media containing the appropriate concentration of drug was added. Cell viability and total viable cells were determined on the seventh day.

The inhibition of HIV-1 p24 antigen production in peripheral blood mononuclear cells (PBMC) by several agents has been studied. Neplanocin-A, 4'-thioadenosine and 5-aza-cytidine were all tested for their anti-HIV activity (See Table I). All clinical HIV-1 isolates were obtained in accord with the methods described above. The HIV tissue culture infectious dose 50% (TCID₅₀) was determined as described previously.

Table I

	<u>IC₅₀</u>		<u>(μM)</u>		
<u>HIV ISOLATE</u>	<u>AZT</u>	<u>NEPA</u>	<u>SAdo</u>	<u>5-aza-c</u>	
pre-AZT (A012)	0.0213	0.0092 (163)	0.0928	0.1211	
post-AZT(A012)	2.1345	0.0012 (1250)	0.6323	0.1733	
re-AZT (A018)	0.0101	0.0122 (125)	0.0932	0.1002	
post-AZT(A018)	1.2325	0.0008 (1875)	0.0645	0.8342	
18199**	1.6456	0.0096 (156)	0.2122	1.2378	
18190**	0.0433	0.0045 (333)	0.1074	0.2134	

NEPA = neplanocin-A

SAdo = 4'-thioadenosine

5-aza-C = 5-aza-cytidine

* Were obtained from National Institutes of Health AIDS Research and Reference Reagent Program.

() gives therapeutic index (IC₅₀ for PBMC cytotoxicity/IC₅₀ for anti-HIV-i activity).

** Exposure to AZT for unknown duration.

Similarly, studies were done evaluating effect of deazaneplanocin (DZNep), deaza-aristomycin and deaza-adenosine, as shown in Table II.

Table II

	<u>IC₅₀</u>		<u>(μM)</u>		
<u>HIV ISOLATE</u>	<u>AZT</u>	<u>DZNep</u>	<u>DZAri</u>	<u>DZA</u>	
pre-AZT (A012)	0.0163	0.0095 (95)	0.1597 (6)	0.1909 (5)	
post-AZT(A012)	2.0729	0.0012 (750)	0.3970 (2)	0.1038 (7)	
re-AZT (A018)	0.0252	0.0153 (59)	0.1867 (5)	0.2187 (3)	
post-AZT(A018)	2.2881	0.0051 (177)	0.0838 (11)	0.6254 (1)	
18199	2.1840	0.0112 (80)	0.1723 (5)	0.9241 (1)	
18190	0.0451	0.0068 (132)	0.0653 (14)	0.1295 (5)	

DZNep = 3-deazaneplanocin

DZAri = 3-deaza-aristeromycin

DZA = 3-deaza-adenosine

It was found, surprisingly, that the HIV strains which were obtained from patients who had been exposed to AZT were unexpectedly susceptible to inhibition by active agents of the invention. The reason for this is not known. However, it is clear that these agents are particularly effective for inhibiting HIV activity of organisms already exposed to AZT.

Compositions for therapeutic use:

Compositions of the invention may be administered by mouth or parenterally. However, when the condition being treated is chronic in nature, it is economically advantageous to administer the drug either in tablet or capsule form or through the mucous membrane. Compositions for administration to the mucous membranes may advantageously be delivered as a mist to the membranes of the respiratory tract or may be administered buccally or sublingually. The active agents may be administered as cyclodextrin inclusion complexes prepared in accord with the teaching of U.S. Patent 4,727,064, which is incorporated herein by reference. Compositions may also be administered rectally as suppositories. The active agents may be formulated in liposomes, microcrystals or microdroplets. For parenteral administration, the usual carriers, including saline, may be used. Other microbials may be administered in conjunction with neplanocin-A. For example, homocysteine or homocysteine thiolactone may be administered to enhance anti-HIV activity.

Compositions of the invention may be administered parenterally by, for example, intramuscular, subcutaneous, or intravenous routes. Dosage of from 0.01 to 4 mg/Kg/day should be administered. Higher dosages may be required early in the treatment program to raise blood levels to effective levels. Dosage of .02 to .5 mg/Kg/day would be more usual. The following examples are provided as examples only and are not intended as limitations.

For oral administration:

Neplanocin-A	50 mg
lactose	80 mg
corn starch	10 mg

The resulting formulation may be placed in a capsule or formed into a tablet for oral administration.

Syrup

	per 30 ml
4'-thioadenosine	50 mg
sucrose	10 gr
sodium benzoate	10 mg
water	qs. to 30 ml

Cyclodextrin inclusion complex

3-deazaneplanocin	50 mg
2-hydroxy- β -cyclodextrin	500 mg
water	qs. to 5 ml.

For oral administration:

3-deaza-aristeromycin	50 mg
lactose	80 mg
corn starch	10 mg

The resulting formulation can be placed in a capsule for oral administration or be formed into a tablet.

Syrup

	per 30 ml
3-deaza-aristeromycin	50 mg
sucrose	10 gr
sodium benzoate	10 mg
Water	qs. to 30 ml

Cyclodextrin inclusion complex

3-deaza-aristeromycin	50 mg
2-hydroxy- β -cyclodextrin	500 mg
water	qs. to 5 ml.

It is understood that the dosage required will depend on the age, size and condition of the patient. The compositions containing the active agents may be administered intravenously in the usual carriers such as normal physiological saline, lactated Ringer's solution, or 5% dextrose in water.

The compositions may also be administered intraocularly. Many AIDS patients suffer from cytomegalovirus as a secondary infection. The compositions of the invention may be particularly useful for administration to these patients. Dosages may be determined by the method described which gave rise to

the data in Table I and II. It is possible thereby to evaluate the susceptibility of the HIV strains that infect patients and to deliver a sufficient dosage to reach the required concentration for inhibition HIV. The suggested initial dosage is that amount required to deliver a blood concentration of 2X to 3X the IC_{50} determined as described above.

Claims:

- 5 1. A human immunodeficiency virus inhibiting formulation containing as an active agent a immunodeficiency virus inhibiting effective amount of at least on active agent selected from neplanocin-A, 4'thioadenosine, 5-azacytidine, 3-deaza-aristeromycin and 3-deazaneplanocin in a pharmaceutical carrier
- 10 2. A formulation of claim 1 wherein the amount of active agent is between .01 mg/kg/day and 4 mg/kg/day.
- 15 3. A formulation of claim 2 wherein the amount of active agent is between .01mg/kg and .5 mg/kg/day.
- 20 4. A formulation of claim 1 which is in capsule form.
5. A formulation of claim 1 wherein the composition is a primarily water.
- 25 6. A formulation comprising a cyclodextrin inclusion complex containing as an active agent at least one compound selected from neplanocin-A, 4'thioadenosine, 5-azacytidine, 3-deaza-aristeromycin and 3-deazaneplanocin.
7. A formulation of claim 1 wherein the active agent is 3-deaza-aristeromycin.
- 30 8. A formulation of claim 1 wherein the active agent is 3-deazaneplanocin.
9. A formulation of claim 1 which is a tablet.
- 35 10. A method of claim 1 wherein the composition is administered intraocularly.
11. A formulation of claim 1 wherein the active agent is

neplanocin-A.

12. A formulation of claim 1 wherein the active agent is 4'thio-adenosine.
13. A formulation of claim 1 wherein the active agent is 5-aza-cytidine.
14. A formulation of claim 1 containing homocysteine or homocysteine lactone and at least one active agent selected from among neplanocin-A, 4'thioadenosine, 5-aza-cytidine, 3-deaza-aristeromycin and 3-deazaneplanocin.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US94/04436

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) : A61K 31/70

US CL : 514/043, 261, 303

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/043, 261, 303

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

FILE CA; BIOSIS; TERMS: "NEPLANOCIN - A", "3 -DEAZANEPLANOCIN", "3 -DEAZAADENOSINE", "4 - THIOADENOSINE" AND "5 - AZACYTIDINE" AND "HIV"

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	BIOCHEMICAL PHARMACOLOGY, Vol. 38, No. 11, issued 01 June 1989, DeClercq et al., "Homocysteine Potentiates the Antiviral and Cytostatic Activity of Those Nucleoside Analogues that are Targeted as S-Adenosylhomocysteine Hydrolase," pp. 1771-1776, see entire document.	14
A	US, A, 5,039,689 (DALUGE ET AL.), 13 August 1991, see entire document.	1-5 and 7-14
X	ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Vol. 33, No. 8, issued August 1989, DeClercq et al., "Broad-Spectrum Antiviral Activities of Neplanocin A, 3-Deazaneplanocin A, and Their 5'-Nor Derivatives," pp. 1291-1297, see entire document.	1-5 and 7-14

☒ Further documents are listed in the continuation of Box C.
 ☐ See patent family annex.

* Special categories of cited documents:	* Later documents published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
* A* document defining the general state of the art which is not considered to be of particular relevance	* X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
* E* earlier document published on or after the international filing date	* Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
* L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	* A* document member of the same patent family
* O* document referring to an oral disclosure, use, exhibition or other means	
* P* document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search 09 AUGUST 1994	Date of mailing of the international search report 16 SEP 1994
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer L. Eric Crane <i>D. Krzyza</i> Telephone No. (703) 308-0196

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/04436

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	MUTATION RESEARCH, Vol. 208, issued 1988, Rascati, "Effects of Cytidine Analogs on Methylation of DNA and Retrovirus Induction," pp. 21-25, see entire document.	1-5 and 7-14
X	J. MED. CHEM., Vol. 35, No. 3, issued 1992, Secrist III et al., "Synthesis and Anti-HIV Activity of 4'-Thio-2',3'-dideoxynucleosides," pp. 533-538, see entire document.	1-5 and 7-14
X	J. Med. Chem., Vol. 36, No. 15, issued 1992, Secrist III et al., "Synthesis of 5'- Substituted Analogues of 3- deazaadenosine as Potential Antivirals", pages 2102 - 2106, see entire document.	1-5 and 7-14
X	FASEB J., Vol. 3, No. 4, issued 19 March 1989, Walker et al., "5-Azacytidine and 5-Azadeoxycytidine Inhibit HIV Replication In Vitro," page A1117, Abstr. No. 5176, see entire abstract.	1-5 and 7-14
A	GB, A, 2,200,651, AL-SUMIDAIE, 10 AUGUST 1988, see attached Abstract No. 111987g.	1-5 and 7-14
X	JP, A, 56-51414, TOYO JOZO, 09 May 1981, pp. 1-9, see attached Abstract No. 175719x.	1-5 and 7-14
X	ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Vol. 34, No. 2, issued February 1990, Bouchard et al., "5 -Azacytidine and 5- Azadeoxycytidine Inhibit Human Immunodeficiency Virus Type 1 Replication in Vitro", pp. 206-209, see entire document.	1-5 and 7-14

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/04436**Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)**

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees.

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING

This ISA found multiple inventions as follows:

- I. Claims 1-5 and 7-14, drawn to a pharmaceutical composition wherein the active ingredient is selected from neplanocin-A, 4'-thioadenosine, 5-azacytidine, 3-deazaaristeromycin and 3-deazaneplanocin and the methods of treating HIV using each of the instant pharmaceutical compositions, classified in Class 514, subclasses 261, 261, 043, 303 and 303, respectively.
- II. Claim 6, drawn to a complex of cyclodextrin and an active agent selected from neplanocin-A, 4'-thioadenosine, 5-azacytidine, 3-deazaaristeromycin and 3-deazaneplanocin, classified in Class 514, subclasses 261, 261, 043, 303 and 303, respectively.

Inventions I and II are related as mutually exclusive species. In the instant case the first invention is directed to pharmaceutical compositions and methods of treating HIV, and the second invention is directed to complexes of cyclodextrin with each of five different nucleoside analogs. Each invention is deemed to be independently useful since there is nothing on the record to show them to be obvious variants. Should applicant traverse on the ground that the species are not inventively distinct from one another, applicant should submit such evidence or identify such evidence now of record showing the species to be obvious variants or clearly admit on the record that this is the case. In either instance, if the examiner finds one of the inventions anticipated by the prior art, the evidence or admission may be used in a finding of lack of inventive step of the other inventions. Accordingly, the claims are not so linked by a special technical feature within the meaning of PCT Rule 13.2 so as to form a single general inventive concept.